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Designer lignins: harnessing the plasticity of lignification

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Lignin is a complex polyphenolic constituent of plant secondary cell walls. Inspired largely by the recalcitrance of lignin to biomass processing, plant engineering efforts have routinely sought to alter lignin quantity, composition, and structure by exploiting the inherent plasticity of lignin biosynthesis. More recently, researchers are attempting to strategically design plants for increased degradability by incorporating monomers that lead to a lower degree of polymerisation, reduced hydrophobicity, fewer bonds to other cell wall constituents, or novel chemically labile linkages in the polymer backbone. In addition, the incorporation of value-added structures could help valorise lignin. Designer lignins may satisfy the biological requirement for lignification in plants while improving the overall efficiency of biomass utilisation.

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Introduction

Lignin is a complex polyphenolic constituent of the secondary cell walls of vascular plants, accounting for 18–35% of the biomass by weight [1]. It is a crucial element of water conduction and plant defence systems in tracheophytes, and it contributes significantly to the compressive strength of secondary xylem tissues. Consequently, lignification of the plant vascular system represents an important evolutionary milestone for land plants. Lignin is one of the most abundant biopolymers on the planet and is an immensely important global carbon sink. However, the chemical recalcitrance of lignin poses a major challenge for industrial biomass processing, most notably in pulp and paper production and in the emerging cellulosic biofuels industry [2,3]. In addition, the lignin content of forage crops is an important consideration in animal nutrition and feed conversion rates in agriculture [4].

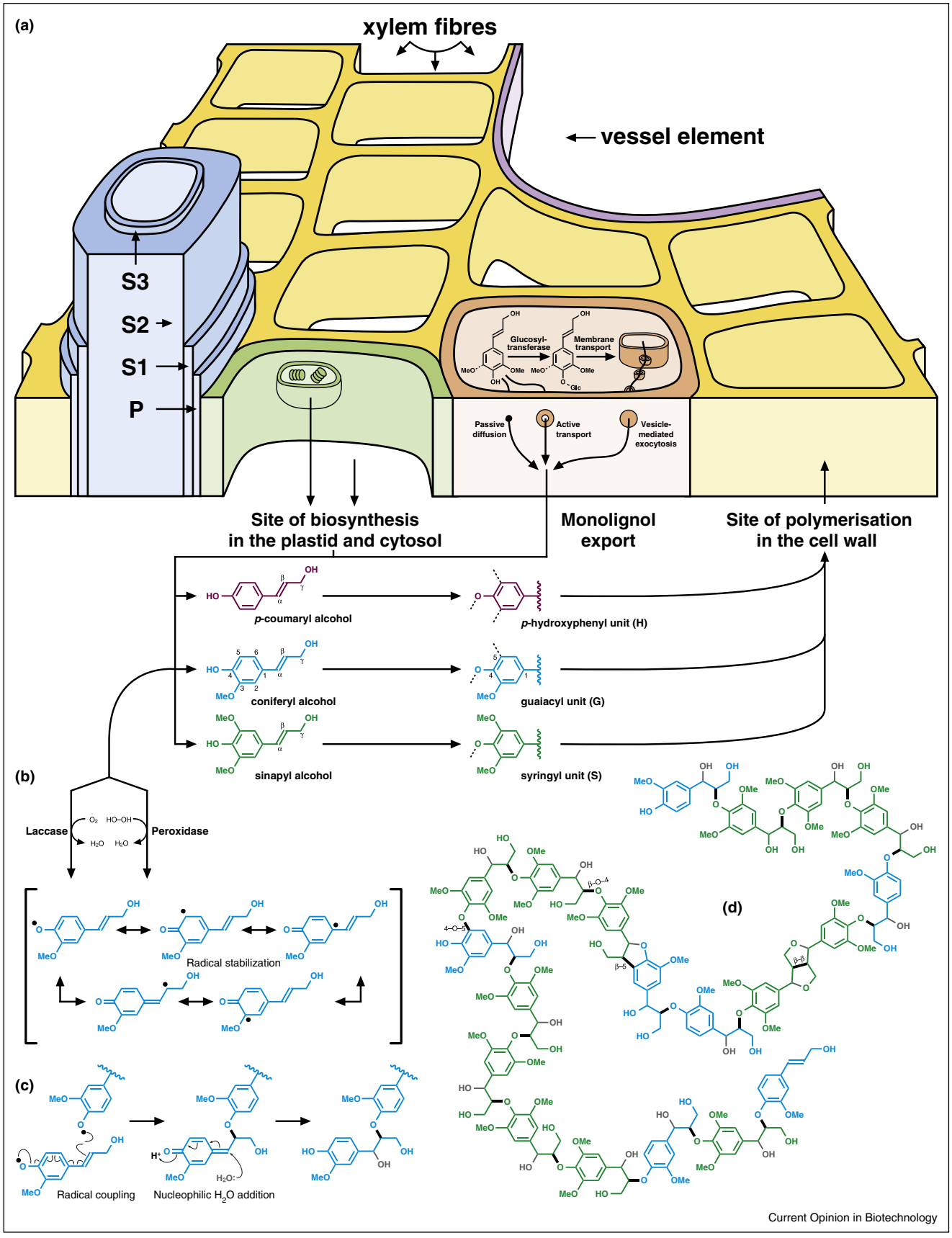
Polymeric lignin is constructed primarily from three 4-hydroxyphenylpropanoids known as monolignols — *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol — that differ only in the degree of aromatic ring methoxylation (Figure 1) [5]. Once incorporated into a lignin polymer, they produce *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) moieties. These canonical monolignols are synthesised in the cytoplasm prior to export to the site of polymerisation in the cell wall where laccase and peroxidase enzymes generate monolignol radicals by dehydrogenation, either through direct action on the monolignols or via a redox shuttle [6]. The incorporation of monolignols into a growing lignin polymer via the combinatorial coupling of radicals results in highly variable racemic polymers with different physicochemical features [7]. For example, gymnosperm lignin is more resistant to degradation largely because it is composed primarily of G subunits that yield abundant β–5, 5–5, and β–β carbon–carbon bonds in addition to the less resilient ether-type β–O–4 linkages that feature more prominently in the S-rich lignins of angiosperms. The amount, composition, and structure of lignins are highly diverse across plant taxa, cell types, developmental stages, and across the cell wall layers. Lignin is embedded in the cell walls of the plant vasculature, notably in the xylem fibres and vessel elements that constitute the bulk of secondary xylem tissues, but also in the sclerenchyma fibres and sclereids in xylem and phloem, and in the cortex cells of the periderm.

Lignin recalcitrance has received considerable research attention and recent advances in our understanding of lignin biogenesis have provoked novel approaches in plant biotechnology [8–10]. In this review, we summarise the progress in lignin engineering, highlight developments within the past three years in the area of designer lignins, and chart a course forward to producing less recalcitrant or more valuable lignins thereby highlighting the potential to enhance the overall utility of this abundant natural polymer.

Lignin pathway engineering

Monolignol biosynthesis occurs via the shikimate and general phenylpropanoid pathways prior to export and

Figure 1


(a)
xylem fibres
vessel element
S3
S2
S1
P
Glucosyl-transferase
Membrane transport
Passive diffusion
Active transport
Vesicle-mediated exocytosis
Site of biosynthesis in the plastid and cytosol
Monolignol export
Site of polymerisation in the cell wall
p-coumaryl alcohol
p-hydroxyphenyl unit (H)
coniferyl alcohol
guaiacyl unit (G)
sinapyl alcohol
syringyl unit (S)
(b)
Laccase
Peroxidase
Radical stabilization
(c)
Radical coupling
Nucleophilic H2O addition
(d)
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deposition into the plant cell wall (see Figure 2 for a detailed overview of the biosynthetic pathways including enzyme abbreviations) [5]. Seven enzyme-catalysed steps of the plastid-localised shikimate pathway convert photosynthate-derived phosphoenolpyruvate and erythrose 4-phospate into chorismate, and further transformations yield the aromatic amino acids phenylalanine, tyrosine and tryptophan. Phenylalanine is then shuttled to the cytosol where the first dedicated step of the general phenylpropanoid pathway is its deamination to produce cinnamic acid. Thereafter, an intricate succession of aromatic ring hydroxylations and catechol unit *O*-methylations interspersed with the activation of cinnamate as a CoA thioester and subsequent reduction via the aldehyde eventually yield the monolignols. There are numerous inter-species variations in the architecture of the lignin biosynthetic pathway, particularly in the preferred route through the metabolic grid. For example, grasses are uniquely capable of using tyrosine in addition to phenylalanine, and gymnosperms are generally unable to produce sinapyl alcohol because the requisite hydroxylase is absent [11].

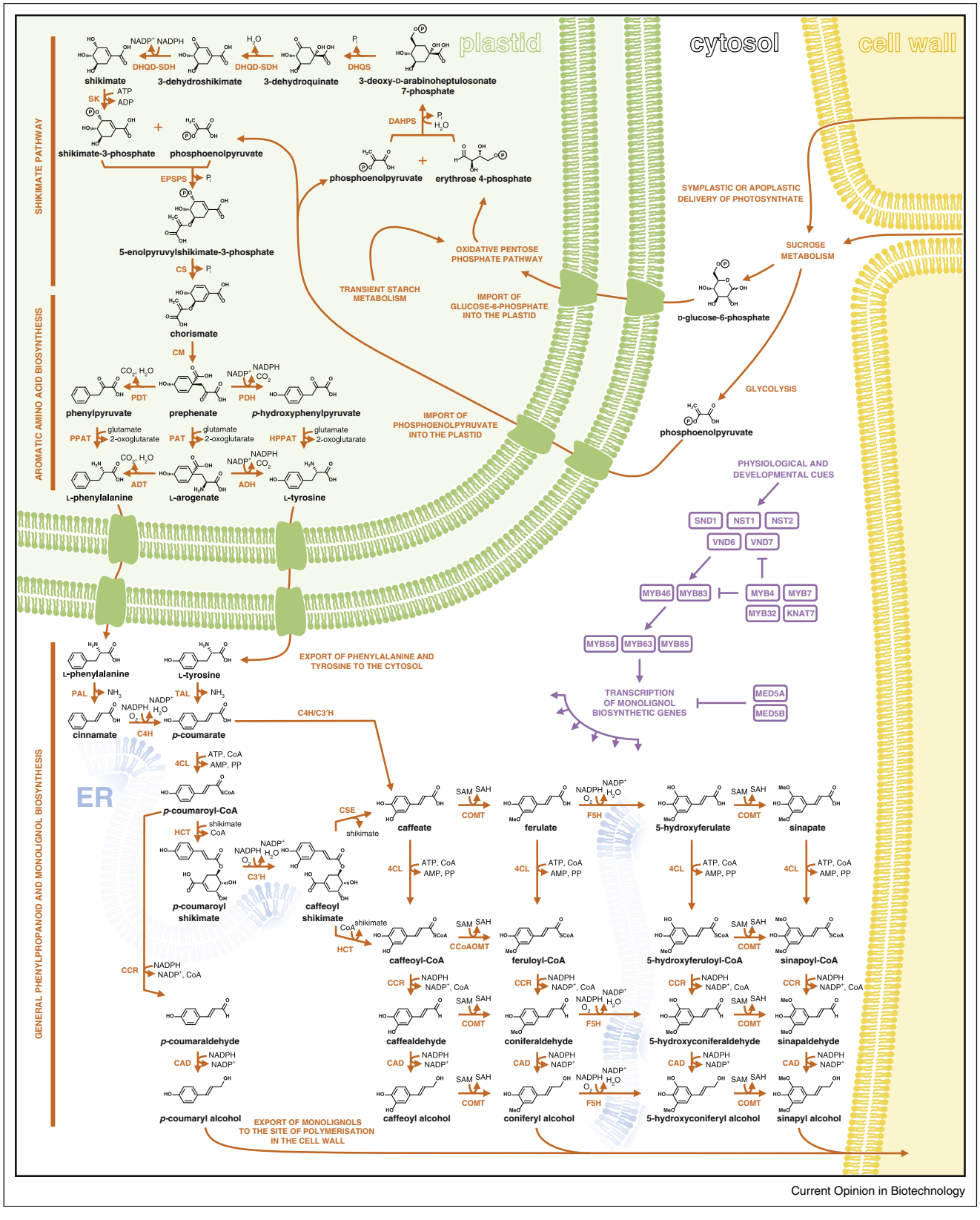
Lignin engineering efforts were initially focused largely on the enzymes directly involved in the general phenylpropanoid and monolignol biosynthetic pathways. Mutants or transgenics with targeted downregulation of key biosynthetic genes in diverse plant species have shown varying levels of reduced lignin production [8]. However, monolignol biosynthesis is highly plastic, allowing plants to substitute monolignols when one or more of the genes is disrupted or misregulated such that lignin composition and quantity are often concomitantly altered. For example, suppression of C3′H in hybrid poplar resulted in a 60% reduction in lignin as well as a shift toward H units [12], whereas knockdown of *PAL* in Brachypodium led to 43% less lignin and a relative increase in S and H units [13]. On the other hand, the Arabidopsis *fah1* mutant lacks a functional F5H, corresponding to a near-complete loss of S units but no significant change in total lignin content [14]. As lignin structure is a reflection of the monomers available during polymerisation and the prevalence of different linkage types is a major determinant of chemical resilience, lignin composition represents an important parameter in biomass recalcitrance that rivals finite lignin content. For instance, the S to G lignin ratio in transgenic hybrid poplar lines has been positively correlated with cell-wall bioconversion rates [15].

Non-canonical monolignols are also amenable to lignification, further emphasising the plasticity of lignin biogenesis. In transgenic poplar for example, downregulation of *CCR* has been shown to lead to the low-level incorporation of ferulic acid [16], downregulation of *CAD* results in the incorporation of hydroxycinnamaldehydes [17,18], and a reduction in *COMT* expression leads to a lignin derived, in part, from 5-hydroxyconiferyl alcohol [19]. Similarly, suppression of *CCoAOMT* in transgenic pine cell cultures leads to the incorporation of caffeyl alcohol [20]. Interestingly, the seed coats of *Vanilla planifolia* and several Cactaceae species contain a lignin derived almost entirely from this monomer or the related 5-hydroxyconiferyl alcohol [21•,22]. More evidence of lignin plasticity is offered by the incorporation of γ-acylated monolignol conjugates, namely acetylated monolignols in kenaf, sisal, palm and abaca, *p*-hydroxybenzoylated monolignols in poplar, willow and palm, and *p*-coumaroylated monolignols in the commelinid monocots [23]. In effect, any compatible phenolic compounds present at the site of polymerisation in the cell wall are 'candidates' for radical transfer, radical–radical coupling, and lignin polymerisation [24]. Although covalent bonds have been shown to link lignin and some hemicelluloses, most notably via the dehydrodimerisation of ferulate on arabinoxylans in grasses [25], the direct attachment of lignin to hemicellulose remains difficult to authenticate [26]. Similarly, cell wall proteins could also become covalently bound to lignin, but this too remains largely unexplored [27].

There has been remarkable progress in altering the amount and composition of lignin by targeting the general phenylpropanoid and monolignol biosynthetic pathways, but lignin-modified plants often exhibit developmental defects ranging from stem lodging to dwarfism. It may simply be that reduced lignin content alters structural integrity and impairs water transport, and such plants often have collapsed xylem cells [28]. However, perturbations to the biosynthetic pathway may also result in the overproduction of other phenylpropanoids and glycoside derivatives that provoke diverse pleiotropic effects [29]. Besides lignin, the shikimate and phenylpropanoid pathways also give rise to an array of other primary and secondary metabolites. Moving forward, lignin engineering strategies

(Figure 1 Legend) Overview of plant cell wall lignification. **(a)** Lignin is produced primarily in the secondary-thickened cell wall layers of xylem tissues; shown here are several xylem fibres and part of a vessel element, shown in purple. The blue-highlighted cell depicts the primary (P) and three secondary cell wall layers (S1, S2 and S3). Biosynthesis of the three canonical monolignols occurs in the cytoplasm, depicted in the green-highlighted cell. Finally, three proposed models of monolignol export to the cell wall are shown in the brown-highlighted cell and polymerisation of monolignols occurs within the cell wall, highlighted in yellow. **(b)** Laccase and peroxidase enzymes present in the cell wall generate monolignol radicals that are stabilised by electron delocalisation (shown for coniferyl alcohol) prior to radical coupling reactions. **(c)** The major polymerisation reaction is the end-wise coupling of a monolignol radical, invariably at its β-position, with the radical of the phenolic end-unit of the growing polymer (shown for the β–O–4-coupling of coniferyl alcohol with a guaiacyl radical). **(d)** An example of a typical polymer model, derived from 20 monolignols, for poplar lignin. For an explanation of colour coding, see the caption for Figure 3.

Figure 2



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should contemplate the effects of altered metabolic flux on related pathways and metabolites. Moreover, rewiring these metabolic networks will be an important element of innovative strategies that incorporate alternative monomers into designer lignins.

Designer lignins

It is now eminently feasible to produce genetically engineered plants with severely reduced lignin levels delivering improved biomass processing; however, such plants are often less vigorous and agronomically inferior [28,29]. By engineering the chemical structure of lignin without drastically altering lignin content or functionality, it may be possible to satisfy the biological requirement for lignification while concomitantly reducing recalcitrance. Novel physicochemical properties could render lignin more easily extractable during processing and could even create new avenues in biomass utilisation [30•]. At least five types of these designer lignins have been proposed: (A) lignins with a lower degree of polymerisation, (B) lignins that are less hydrophobic, (C) lignins with fewer bonds to structural carbohydrates, (D) lignins containing chemically labile bonds, and (E) lignins designed to harbour value-added chemical moieties (Figure 3). Several examples of these designer lignins will be described here to illustrate the breadth of possibilities.

A. Shorter lignin chains

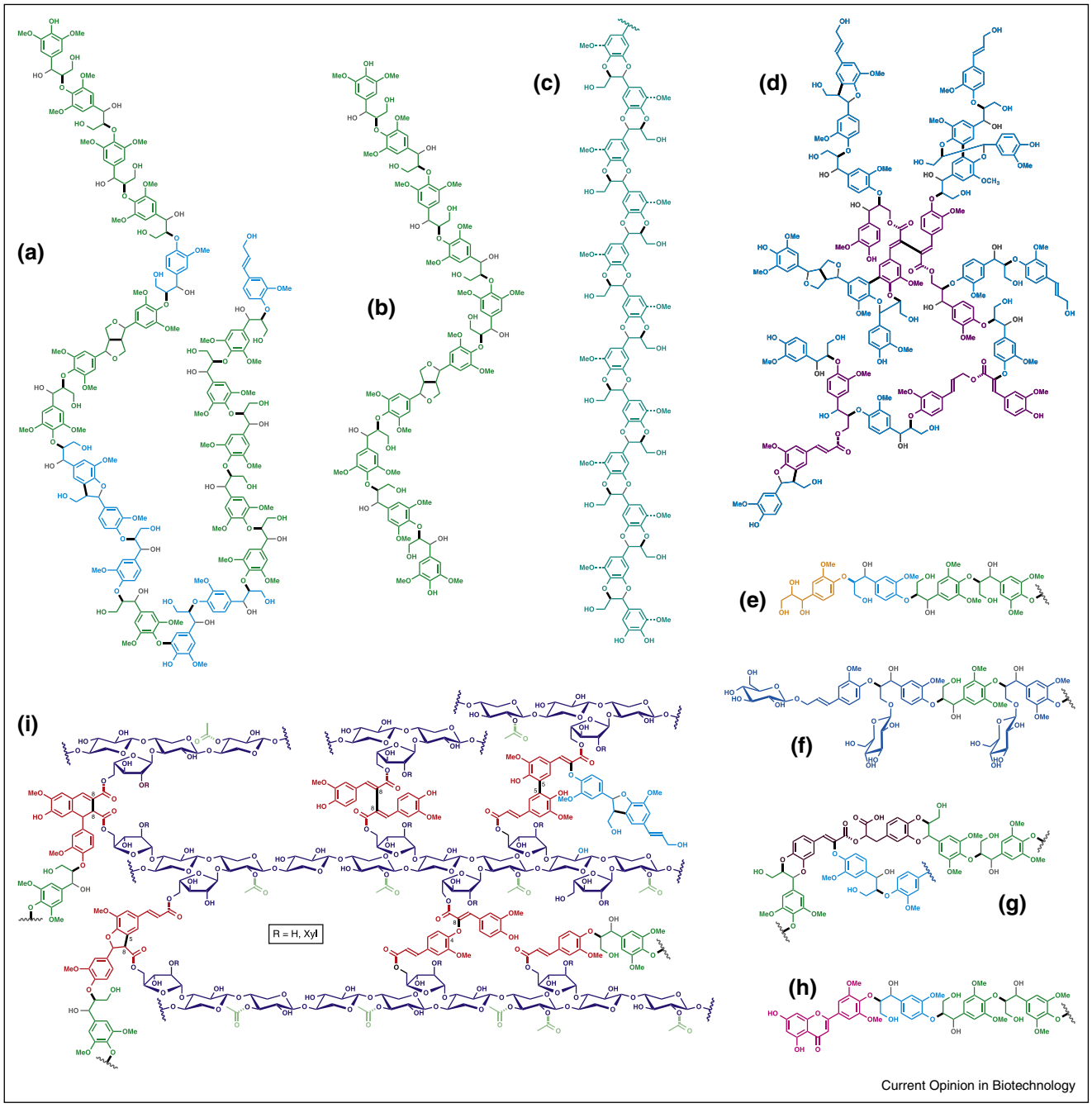
Although conventional wisdom maintains that lignin polymers have a high molecular weight and are extensively cross-linked, recent evidence suggests that native lignin comprises relatively short oligomeric chains with only minimal branching [31]. Nonetheless, lignins with reduced degrees of polymerisation may be more readily extracted. Incorporation of monomers capable only of single coupling reactions that either initiate ('starters') or terminate ('stoppers') chain elongation could reduce average lignin polymer chain lengths. For example, dihydroconiferyl alcohol found in the lignin of gymnosperms [17], as well as hydroxyphenylglycerols (Figure 3e) and hydroxybenzenoids detected in a variety of plants, can serve as lignin initiators [7,32••]. A greater prevalence of these groups during active polymerisation may lead to more polymer initiation events resulting in more lignin chains that are shorter. Consistent with this approach, expression of the bacterial hydroxycinnamoyl-CoA hydratase-lyase gene in Arabidopsis lignifying cells did not alter plant growth or overall lignin content but did lead to side-chain shortening of monomers, the incorporation of *p*-hydroxybenzaldehyde and *p*-hydroxybenzoate groups, reduced lignin chain length, and improved saccharification [32••]. An analogous tactic is to exploit the relative oxidation potential of different monomers in an effort to introduce more end-groups. For example, *p*-coumaryl alcohol favours radical transfer reactions and frequently occurs as free-phenolic endgroups such that H-rich lignins have a lower degree of polymerisation [33]. The S-rich lignin from transgenic poplar overexpressing *F5H* also appears to have a remarkably shorter chain length with an average degree of polymerisation of approximately 10 (Figure 3b) [34].

B. Less hydrophobic lignins

Lignin is largely hydrophobic in nature and designer lignin polymers containing more hydrophilic constituents could be more soluble during delignification processes. Additionally, increased hydrophilicity could reduce hydrophobic interactions in the cell wall and improve enzyme accessibility during saccharification. Various alternative strategies are conceivable and candidate monomers may contain additional hydroxyl groups or conjugated hydrophilic moieties, such as carbohydrates [35] (e.g., Figure 3e–g). The opposite strategy could be favourable; molecular dynamics simulations of lignin with increased hydrophobicity predicted a reduction of non-covalent associations between lignin and hemicelluloses [36] that could make the cell wall polysaccharides more accessible during saccharification.

(Figure 2 Legend) Biosynthesis of monolignols via the shikimate, aromatic amino acid, and phenylpropanoid pathways. Photosynthate, supplied as sucrose and stored transiently as starch, is metabolised in sink tissues via glycolysis and the oxidative pentose phosphate pathway to produce phosphoenolpyruvate and erythrose 4-phosphate. These in turn are converted via the shikimate pathway into chorismate. The aromatic amino acid pathway yields phenylalanine, tyrosine and tryptophan (not shown). Finally, the monolignols, primarily *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, are produced through the general phenylpropanoid pathway and the monolignol-specific biosynthetic pathway. Enzymatic reactions and metabolite shuttling are shown in orange, the plastid as well as membrane transporters and the inner and outer plastid membranes are coloured in green, the ER and ER membranes are depicted in blue, and the plasma membrane and cell wall are highlighted in yellow. Note that not all routes shown have been demonstrated in all plants; for example: *TAL* has been found only in monocots, *CSE* activity has only been demonstrated in Arabidopsis so far, *F5H* is absent from most gymnosperms, and the route from *p*-coumarate directly to caffeate has only been demonstrated in poplar. The cascade of transcriptional regulation is shown in purple and includes proteins from the MYB, KNAT and NAC families of transcription factors as well as two subunits of the Mediator transcriptional co-regulator complex. *Enzyme abbreviations*: 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHPS), 3-dehydroquinate synthase (DHQS), 3-dehydroquinate dehydratase–shikimate dehydrogenase (DHQD-SDH), shikimate kinase (SK), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), chorismate synthase (CS), chorismate mutase (CM), prephenate aminotransferase (PAT), arogenate dehydrogenase (ADH), arogenate dehydratase (ADT), prephenate dehydrogenase (PDH), 4-hydroxyphenylpyruvate aminotransferase (HPPAT), prephenate dehydratase (PDT), phenylprephenate aminotransferase (PPAT), phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL), cinnamate-4-hydroxylase (C4H), 4-coumarate CoA ligase (4CL), *p*-hydroxycinnamoyl-CoA: shikimate/quinate *p*-hydroxycinnamoyltransferase (HCT), *p*-coumaroyl-shikimate/quinate-3-hydroxylase (C3′H), caffeoyl shikimate esterase (CSE), caffeoyl-CoA *O*-methyltransferase (CCoAOMT), ferulate/coniferaldehyde-5-hydroxylase (F5H), caffeic acid/5-hydroxyconiferaldehyde *O*-methyltransferase (COMT), cinnamoyl-CoA reductase (CCR), and cinnamyl alcohol dehydrogenase (CAD). *Other abbreviations*: inorganic phosphate (P_i), S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH).

Figure 3



Lignin models and designer lignins. **(a)** A poplar lignin model containing 20 units, S:G = 13:7. 4–O–5-Coupling was thought to produce branching, but this is now being questioned [31]; the unit (near the bottom of the structure) is shown here as a free-phenolic unit; *p*-hydroxybenzoates acylating some γ-OH groups are not shown. **(b)** An all-S poplar lignin model, showing only the two types of primary units, β–β and β–O–4; such lignins are essentially linear and may have a low degree of polymerisation (i.e., are chain-shortened) [34]. **(c)** Almost completely homogeneous (in terms of interunit linkage type) and linear lignins are produced, *in vivo* and *in vitro*, from atypical monolignols caffeyl alcohol (no 5-OMe) and 5-hydroxyconiferyl alcohol [21,22]; such lignins are also devoid of lignin–polysaccharide cross-linking as rearomatisation of the quinone methide intermediate following β–O–4-coupling is via fast internal trapping by the *o*-phenol. **(d)** A model of a high-zip lignin from 16 coniferyl alcohol monomers and 4 coniferyl ferulate conjugates (magenta) showing some of the ways that both the ferulate and the monolignol moiety may couple into the polymer to form a potentially more cross-linked polymer that nevertheless readily falls apart during pretreatment [23,39••]; readily cleavable ester bonds are shown hashed. Cleaving this oligomer containing 4 zip-conjugates cleaves this model lignin into 5 fragments; in general, a polymer containing *n* zip-conjugates will cleave into $\leq(n+1)$ fragments. **(e)** A fragment of a chain-shortened polymer created by lignification using monomers, such as the guaiacylglycerol shown here, that can only start a lignin chain; this polymer is logically also more hydrophilic. **(f)** A fragment of a particularly hydrophilic lignin polymer created in part using monolignol γ–O–1β-glucosides *in vitro* [52]. **(g)** A fragment of lignin

C. Lignins with less structural cross-linking to carbohydrates

In addition to non-covalent interactions, lignin is conjectured to be linked to hemicelluloses through various types of covalent bonds. Whenever a monolignol couples via its β position to the growing polymer (or to another monomer), a quinone methide intermediate is produced and, in the case of the dominant β-ether units, this is overwhelmingly quenched and re-aromatised via nucleophilic attack from water (Figure 1c) [7]. Alcohol and carboxylic acid groups inherent to hemicelluloses may also theoretically serve as nucleophiles giving rise to the benzyl ether or benzyl ester bonds that contribute to recalcitrant lignin–carbohydrate complexes, but compelling evidence for this is still lacking. Potentially useful novel units containing *o*-diphenol groups, such as those arising from caffeyl alcohol or 5-hydroxyconiferyl alcohol, result in quinone methide intermediates that are rapidly internally trapped, forming benzodioxane structures (Figure 3c) before any possibility of external nucleophilic attack can occur. A number of similar candidate monomers that could reduce polysaccharide–lignin cross-linking have recently been validated in biomimetic *in vitro* studies, including rosmarinic acid, epicatechin, ethyl gallate and epigallocatechin [35,37]. As noted above, monocots have a distinctive mechanism for cross-linking cell wall polymers to strengthen the wall; arabinoxylan is acylated with ferulate moieties that can radically cross-couple to adjacent feruloylated hemicellulose chains or to lignin resulting in extensive polysaccharide–polysaccharide and polysaccharide–lignin cross-linking (Figure 3i) [23]. Several groups are currently working to identify the acyltransferase enzyme(s) responsible for acylating the arabinosyl units on arabinoxylans with ferulate [38].

D. Lignins with novel chemically labile bonds

As has been shown, various perturbations of the monolignol biosynthetic pathway result in a shift in monomer composition that may yield more chemically labile bonds [5,8,30•]. For example, ferulic acid incorporates into the lignin of *CCR*-deficient plants producing acid-labile acetals [16], and overexpression of *F5H* in hybrid poplar results in a lignin containing nearly 98% S units yielding more alkali-labile β-ethers (Figure 3b) [34]. One of the most highly sought after objectives has been the introduction of S lignin units into conifer tree species that normally contain the more recalcitrant G-rich lignin. Recently, an important proof-of-principle milestone was achieved with the simultaneous introduction of *COMT* and *F5H* genes into transgenic pine cell cultures [11]. The 'zip-lignin' strategy has also been heralded as a major breakthrough in designer lignins [23,39••]. Inspired by the proven incorporation of ferulates integrally into grass lignins, an exotic feruloyl-CoA:monolignol transferase from *Angelica sinensis* was introduced into hybrid poplar and resulted in a lignin in which chemically labile ester bonds had been integrated into the polymer backbone (Figure 3d), improving cell wall digestibility after mild alkaline pretreatment [39••]. Recently, a bacterial Cα-dehydrogenase was shown to oxidise the α-hydroxyl groups in lignin and, when introduced into transgenic Arabidopsis, yielded low levels of novel chemically labile α-keto-β-ether units in lignin [40•]. Finally, as amino acids are also capable of cross-linking to lignin, an alternative approach is to direct tyrosine-rich or cysteine-rich peptides to the cell wall such that protease enzymes could digest these cross-links and accelerate lignin digestion [41].

E. Value-added lignins

Lignin extracted during industrial biomass processing is frequently used for its calorific value, being burnt to provide process energy. But recently, aspirations of complete biomass utilisation within the modern biorefinery have inspired an array of lignin-derived high-value products [42]. Research in this area has primarily focused on optimising lignin recovery and developing products from lignin that can compete with existing petroleum-derived materials. However, moving forward, inherently valuable lignin polymers could be developed to facilitate the production of novel high-value products using industrial lignin waste streams. The abundant aldehyde groups in the above-mentioned hydroxycinnamaldehyde-derived lignins from *CAD*-deficient plants [17,18] create enormous potential for functionalisation in diverse applications. Lignins derived solely from caffeyl alcohol or 5-hydroxyconiferyl alcohol monomers produce homogeneous linear lignin chains of β–O–4-derived benzodioxane units (Figure 3c) [21•,22]. Such regularity would likely be beneficial in applications such as the generation of lignin-derived carbon fibres, due to its homogeneous structure and lower complexity. However, as intriguing as the possibilities are, it is not yet clear that large, healthy plants can be produced with such lignins. Nature herself

(Figure 3 Legend Continued) containing rosmarinic acid (purple) that has been incorporated into the chain via radical coupling [37]. Such lignins would display various features, including being more hydrophilic (due to the acid group), having benzodioxane units (like the polymers in c) that preclude lignin-polysaccharide cross-linking at those sites, and zip-lignin signatures allowing the polymer to be readily cleaved by mild base (the cleavable ester linkage is shown hashed). **(h)** An example of a high-value component in lignin–tricin end-units (magenta) occur in grass lignins [43•]. Tricin is a flavonoid and it is synthesised outside the monolignol biosynthetic pathway. **(i)** Model of the extensive polysaccharide–polysaccharide (via arabinoxylan-bound ferulate dimerisation) and polysaccharide–lignin (via incorporation of the ferulates and diferulates (red, on arabinoxylan) into the lignin polymer) in all commelinid monocots [23,25]; again, readily cleavable ester bonds are shown hashed. a–i. The bonds formed by radical coupling reactions are bolded; bonds formed during post-coupling rearomatisation are grey, as are the OH groups from water addition (see Figure 1c). In all of the models, units derived from lignin monomers are in cyan (G) and green (S), whereas novel units are coloured uniquely.

is revealing pathways by which valued products could naturally be produced in lignins and is even showing how they can be arranged to be on the end of a chain where they are, presumably, easiest to cleave off. For example, it was recently discovered that the flavonoid tricin is naturally incorporated into monocot lignins, as (starting) endgroups (Figure 3h) [43•]. This discovery, further illustrating the inherent plasticity of lignification, was particularly remarkable because tricin is not produced via the monolignol biosynthetic pathway so it exemplifies how, through thoughtful metabolic engineering, it may be possible to incorporate other unique and valuable chemical constituents into lignin. Given the abundance of surplus lignin available in industry, it is plausible that even low-level production of value-added lignins could help economise total biomass utilisation in biorefineries.

Perspectives on lignin engineering

A number of potential alternative monomers has been proposed, many of which have overlapping functionalities in designer lignins [30•]. For example, rosmarinic acid is a hydrophilic compound with a chemically labile ester linkage and two *o*-diphenol groups (Figure 3g) [37]. The resulting lignin polymers would therefore be less hydrophobic, possess fewer links to hemicelluloses, and have readily cleavable ester bonds within lignin chains. Before embarking on plant metabolic engineering with novel lignin monomers, *in vitro* experiments and biomimetic test systems can be used to validate design strategies [35,37]. For example, with rosmarinic acid, these tests revealed no barriers to radical formation or lignin polymerisation in maize cell walls and pointed to significant improvements in saccharification [37]. A number of additional alternative monomers are currently being evaluated through a similar pipeline and plant engineering work with the most promising candidates will soon follow [30•].

The 'zip-lignin' strategy to introduce backbone esters exemplifies the potential of monolignol acyltransferases in lignin engineering [39••]. Presently, genes encoding *p*-coumaroyl-CoA:monolignol transferase and feruloyl-CoA:monolignol transferase have been discovered [39••,44], whereas genes for acetyl-CoA and *p*-hydroxybenzoyl-CoA analogues remain elusive. Recent surprising discoveries in the chemical variability of natural lignins leave room to ponder what additional monolignol acylation possibilities may exist and whether corresponding acyltransferases might be found in nature. Coupled with innovative metabolic pathway engineering to supply these alternative monomers, transformational changes in lignin engineering appear to be within reach.

As the regulation of monolignol biosynthesis is largely enacted through transcriptional control, considerable attention has been devoted to demystifying these processes and harnessing them to actively switch biosynthesis on or off at will. In addition to the array of MYB family transcription factors that activate monolignol biosynthetic genes and the NAC family transcription factors that serve as master switches for secondary cell wall biosynthesis, subunits of the transcriptional co-regulator Mediator have recently been identified as homeostatic repressors of monolignol biosynthesis and all of these show promise in lignin engineering [45,46••] (Figure 2). Although many designer monomers may originate from the phenylpropanoid pathway directly, other monomers may have different metabolic origins and regulatory constraints. It also remains to be seen how designer lignins might ensure adequate cell wall properties in the context of recently proposed cell wall integrity models [29,47]. Although monolignol transport mechanisms remain obscure, recent evidence points to active transport through membrane-bound transporter proteins [48]. The realisation that alternative monomers are also translocated to the site of polymerisation in the cell wall casts some doubt on these concepts. It may be that monolignol transporters are exceptionally non-specific, or perhaps that monolignols are not exported by active transport whatsoever. Similarly, several peroxidase and laccase enzymes have been implicated in the dehydrogenation of monolignols [49]; however, these proteins are also apparently non-selective as non-canonical monomers are routinely incorporated. Alternatively, it may be that these monomers are not accepted directly and rely solely on radical transfer reactions to enter polymerisation. Although there are no obvious barriers for export, radical formation, and incorporation of non-canonical monomers into lignin polymers, these mechanisms represent underexploited avenues in lignin engineering.

Designer lignins invariably possess novel physicochemical properties that could potentially have adverse effects on plant physiology and development. Manipulations of the monolignol biosynthetic pathway have been known to produce plants with reduced growth, developmental defects, increased susceptibility to disease and water transport difficulties [29,50]. Although not all lignin-modified plants are compromised and such widespread pleiotropic phenotypes may not be purely a consequence of reduced lignin content or altered composition, it emphasises the biological importance of lignin in plants. In an effort to produce agronomically viable plants, broader plant metabolism should be considered and it may be prudent to use tissue-specific promoters to drive transgene expression. Spatiotemporal control may restrict designer lignins to xylem tissues, or xylem fibres more specifically [9]. Additionally, yield penalties could perhaps be overcome by vessel-specific complementation [51]. Given the diversity of lignin structures tolerated by plants in nature, there is every reason to believe that some novel designer lignins will be compatible with normal plant growth. Moreover, it is imperative that all these novel transgenic strategies are appropriately field-tested to ensure that the observed trait modifications withstand normal growing conditions.

Conclusions

Lignin engineering has evolved beyond simple perturbations of the general phenylpropanoid and monolignol biosynthetic pathways, culminating in a suite of designer lignins with novel physicochemical properties. Much like the Trojan horse used to covertly circumvent the wall defences of the ancient city of Troy, designer lignins may satisfy the biological requirement for lignin in plant cell walls while providing improved biomass utilisation efficiency. Moreover, high-value designer lignins have been conceived that could valorise lignin waste streams in biomass refineries, and could be targeted toward upgrading processing. Lignification shows remarkable plasticity, but the development of agronomically viable biomass feedstocks featuring designer lignins will require thoughtful selection of non-canonical monomers, validation in biomimetic systems, careful metabolic engineering, thorough assessment of pleiotropic effects that could potentially accrue from the incorporation of novel components, field trials, and the generation of sufficient volumes of material for industrially relevant bioconversion assessment. Although possibilities abound, maintaining plant health is paramount and, ultimately, the plants themselves will dictate which of these approaches can be tolerated. At the dawn of this new era in lignin engineering, we are limited only by the biological constraints of lignification and by our collective imagination.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
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